

1 **Transcutaneous Spinal Direct Current Stimulation** 2 **(tsDCS) Modulates Human Corticospinal System** 3 **Excitability**

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30

31 Abstract

32 This study aimed to assess the effects of thoracic anodal and cathodal transcutaneous spinal
33 direct current stimulation (tsDCS) on upper- and lower-limb corticospinal excitability. Yet,
34 despite studies assessing thoracic tsDCS influences the spinal ascending tract and reflexes,
35 none assessed the effects of this technique over upper- and lower-limb corticomotorneuronal
36 connections.

37 In 14 healthy subjects we recorded motor evoked potentials (MEPs) elicited by transcranial
38 magnetic stimulation (TMS) from abductor hallucis (AH) and hand abductor digiti minimi
39 (ADM) muscles before (baseline, B), and at a different time-points (0 and 30 minutes) after
40 anodal or cathodal tsDCS (2.5 mA, 20 minutes, T9-T11 level). In 8 of the 14 subjects we also
41 tested the soleus H-reflex, the F-waves from AH and ADM before and after tsDCS.

42 Both anodal and cathodal tsDCS left the upper-limb MEPs and F-wave unchanged.
43 Conversely, while leaving lower-limb H-reflex unchanged, they oppositely affected lower-
44 limb MEPs: whereas anodal tsDCS increased resting motor threshold ($\{mean \pm SEM\}$ 107.33
45 \pm 3.3%, increase immediately after tsDCS, and 108.37 \pm 3.2% increase 30 min after tsDCS
46 compared to baseline), and had no effects on MEP area and latency, cathodal tsDCS increased
47 MEP area (139.71 \pm 12.9% increase immediately after tsDCS and 132.74 \pm 22.0% increase 30
48 min after tsDCS compared to baseline) without affecting resting motor threshold and MEP
49 latency.

50 Our results show that tsDCS induces polarity specific changes in corticospinal excitability
51 that last for more than 30 min after tsDCS offset and selectively affect responses in lower-
52 limb muscles innervated by lumbar and sacral motoneurons.

53

54 *Key words:* direct current stimulation; spinal cord stimulation; spinal cord; corticospinal
55 system; tsDCS; tDCS; TMS; motor potentials.

56

57 **Introduction**

58 Transcutaneous spinal direct current stimulation (tsDCS) is a simple, painless and
59 noninvasive technique for modulating spinal cord function in humans (Cogiamanian et al.
60 2012; Cogiamanian et al. 2011; Lamy and Boakye 2013b; Priori et al. 2014). tsDCS consists
61 in delivering a constant direct current (DC) at 1.5-2.5mA over the spinal cord through a pair
62 of sponge electrodes. The technique induces effects lasting from minutes to hours
63 (Cogiamanian et al. 2008; Lamy et al. 2012; Lim and Shin 2011; Winkler et al. 2010) and is
64 well tolerated by subjects. After the first reports (Cogiamanian et al., 2008; Winkler et al.,
65 2010), this non-invasive method for spinal neuromodulation has come into increasingly
66 widespread use (Cogiamanian et al. 2011; Hubli et al. 2013; Lamy and Boakye 2013b).
67 Although different settings and stimulation parameters have been used, the “monopolar”
68 montage (active electrode over the lower thoracic spinal cord, return electrode over the right
69 arm) reduces the spread of the current towards the higher spinal cord levels or to the
70 brainstem (Parazzini et al. 2014). Equally important, longitudinal electrical fields, as those
71 induced by this montage, may have important implications for rehabilitation as they promote
72 axonal regrowth and prevent fiber degeneration (Hernandez-Labrado et al. 2011).

73 In earlier research in our laboratory (Cogiamanian et al., 2008), we found that thoracic
74 anodal tsDCS depresses the cervico-medullary SEP component (P30). tsDCS also modulates
75 post-activation H-reflex dynamics (Winkler et al., 2010; Lamy et al., 2012) and the flexion
76 reflex in the human lower limb (Cogiamanian et al., 2011). Further experiments reported that
77 tsDCS impairs conduction in the ascending nociceptive spinal pathways thus increasing pain
78 tolerance in healthy subjects (Truini et al. 2011). Cervical spinal DC stimulation increased
79 upper-limb muscle motor evoked potential (MEP) amplitudes (Lim and Shin, 2011). The
80 effects induced by tsDCS could arise from the influence of electric field on impulse

81 conduction, membrane excitability, GABAergic and glutamatergic transmission (Priori et al.
82 2014). Whatever the mechanisms, by modulating spinal cord function, tsDCS could provide a
83 novel therapeutic tool complementary to drugs and invasive spinal cord stimulation in
84 managing various pathological conditions, including pain, spasticity and movement disorders.

85 Having more information on the underlying mechanisms is an essential prerequisite
86 for designing future clinical applications and, more specifically, to understand whether and
87 how tsDCS influences the corticospinal system would be relevant. Though interesting, the
88 work of Lim & Shin (2011) on upper limb motor responses cannot rule out possible non
89 specific effects. Hence, to expand the knowledge about the tsDCS effects on the human
90 corticospinal pathways, we tested upper and lower limb MEPs elicited by transcranial
91 magnetic stimulation (TMS) in a group of healthy subjects before and after tsDCS. To
92 evaluate possible motorneuronal or reflex excitability changes induced by tsDCS we also
93 tested the F-wave from the lower limb abductor hallucis (AH) and the upper limb abductor
94 digiti minimi (ADM) muscles and the H-reflex from soleus muscle.

95

96 **Materials and Methods**

97

98 *Transcutaneous spinal DC stimulation (tsDCS)*

99 With participants lying supine on a comfortable couch, tsDCS (2.5 mA, 20 min) was
100 delivered by a constant current programmable electrical stimulator (HDCStimTM, Newronika,
101 Italy) connected to a pair of electrodes, one centred over the spinous process of the 10th
102 thoracic vertebra with the major axis longitudinally placed so that it spanned from 9th to 11th
103 thoracic vertebrae, and the other above the right shoulder on the deltoid muscle (Cogiamanian
104 et al., 2008, 2011). Because the tibial nerve arises from L4 to S3 spinal levels that correspond
105 to 9th to 12th vertebral level, the active tsDCS electrode was placed over lumbar and sacral
106 motorneurons. tsDCS electrodes were thick (6 mm) rectangular pieces of saline-soaked
107 synthetic sponge (7 x 5 cm, 35 cm²). We applied current at a density of 0.071 mA/cm² and

108 delivered a total charge density of 85.7 mC/cm^2 which is below the threshold values for tissue
109 damage (Liebetanz et al. 2009; McCreery et al. 1990). The wide electrode surface avoided the
110 possible harmful effects of high current density. Apart from occasional, transient and short-
111 lasting tingling and burning sensations below the electrodes, tsDCS remained below the
112 conscious sensory threshold throughout the experimental session. tsDCS polarity (cathodal or
113 anodal) refers to the electrode over the spinal cord.

114

115 *Motor evoked potentials (MEPs)*

116 Transcranial magnetic stimulation (TMS) was delivered by a Novamatrix Magstim
117 200 stimulator (Magstim[®], Whitland, Carmarthenshire, UK) through a flat coil (outer
118 diameter 13.5 cm) in which current flows clockwise (viewed from above). The coil was kept
119 in a constant position centered over the vertex for both upper and lower limb; for the upper
120 limb one edge of the coil was slightly tilted towards the hemisphere to be stimulated (Groppa
121 et al., 2012). Motor evoked potentials (MEPs) were recorded at rest by two standard non-
122 polarizable Ag/AgCl surface electrodes (diameter 10 mm; Technomed Europe[®]), one placed
123 over the belly of the *abductor digiti minimi* (ADM) muscle, and the other on the skin
124 overlying the first metacarpophalangeal joint of the fifth finger of the left hand; for lower
125 limbs, MEPs were recorded through one electrode placed over the belly of the *abductor*
126 *hallucis* (AH) muscle and the other on the first metatarsophalangeal joint of the left toe.
127 Because both ADM and AH have been used in many TMS studies in normal subjects and in
128 patients (Chen et al. 1998; Nakanishi et al. 2006; Osei-Lah and Mills 2004), and our
129 laboratory uses both muscles in routine TMS studies, we selected these two muscles for our
130 experiments.

131 Stimulation intensity was set at 120% of resting motor threshold (RMT) defined as the
132 minimum stimulator output that evoked MEPs higher than $50 \mu\text{V}$ in at least five out of 10

133 trials when muscle is completely relaxed (Di Lazzaro et al. 1999; Ni et al. 2007). The
134 threshold was set differently for each muscle and was analyzed at each time point (B, T0,
135 T30). However, to compare MEP modifications across time we used the same stimulation
136 intensity (baseline). A total of ten MEPs were collected at approximately 10 s intervals and
137 averaged for each time point. MEPs were amplified and filtered (bandwidth 3Hz–3kHz,
138 Nicolet Viking IV P). Three different variables were measured: RMT (% of stimulator
139 output), onset latency (ms) and area-under-the curve (mVms) of motor response. RMT was
140 measured before and after tsDCS, MEP area and latency were measured off-line on the MEPs
141 averaged from ten sweeps.

142

143 *H-reflex*

144 H-reflexes were elicited in eight subjects by delivering 1 ms rectangular pulses
145 through Ag/AgCl electrodes (10-mm diameter), placed over the left tibial nerve at the
146 popliteal fossa (inter-electrode distance 20 mm), and recorded from the soleus muscle,
147 through Ag/AgCl electrodes (10-mm diameter) placed 2 cm apart over the muscle belly. The
148 leg was fixed, with the hip semi-flexed ($\sim 110^\circ$), the knee slightly flexed ($\sim 150^\circ$), and the
149 ankle in approximately 10° plantar flexion. The current intensity was progressively increased
150 to obtain H-reflex threshold (defined as the minimum stimulation intensity that evoked
151 reproducible response higher than 50 μ V), maximal H-reflex, and maximal compound muscle
152 action potential (CMAPmax). To avoid post-activation effects, the tibial nerve was stimulated
153 at intervals randomly varying between 10 and 20 s.

154 To define threshold and maximum size of H-reflex, stimulation began at 0 mA intensity and
155 increased in 1 mA steps up to the intensity eliciting the maximal H-reflex. Signals were
156 amplified and band-pass filtered (3Hz–3kHz). We measured the H-reflex size (peak-to-peak
157 amplitude, mV) and we calculated the Hmax/CMAPmax ratio.

158

159 *F-wave*

160 F-waves were elicited in eight subjects with a 25% supra maximal stimulation applied
161 to the tibial nerve and recorded from the abductor hallucis (AH) muscle, or to the ulnar nerve
162 and recorded from the abductor digiti minimi (ADM) muscle through a pair of 10 mm surface
163 Ag/AgCl electrodes in a belly-to-tendon configuration. Tibial-nerve evoked F-waves from the
164 AH muscle were obtained by 20 stimuli delivered to the left ankle and at an interstimulus
165 interval of 1 s (1 Hz). Similarly, ulnar F-waves from ADM muscles were elicited by 20
166 stimuli delivered to the ulnar nerve at the left wrist with an interstimulus interval of 1 s (1
167 Hz). Subjects were asked to fully relax. To ensure the absence of muscular activity we
168 recorded the audio EMG feedback from the same muscles used for MEP recording at all time
169 points. F-wave mean latency (ms), minimal latency (ms), mean amplitude (mV), mean
170 temporal dispersion (ms) were collected and analysed. The filter setting was 15-1500Hz and
171 skin temperature at the ankle and wrist was kept above 32°C.

172

173 *Subjects and experimental procedure*

174 A group of 14 healthy right-handed volunteers (nine women and five men, mean
175 [\pm SD] age 25.6 ± 4.3 years) participated in the study, which was approved by the institutional
176 review board. Before enrollment, the study protocol was explained to each subject and
177 informed written consent was obtained. The experimental procedures were conducted in
178 accordance with the declaration of Helsinki.

179 Subjects were studied before and after anodal and cathodal tsDCS. Cathodal and
180 anodal tsDCS in each subject were tested in random order and at least 1 week elapsed
181 between sessions. The subjects were blinded about tsDCS polarity. Because preliminary
182 experiments showed that anodal and cathodal tsDCS elicited MEP changes in the opposite

183 directions and subjects were unable to discriminate stimulation polarity (as for brain tDCS),
184 as in previous works (Cogiamanian et al. 2008; Truini et al. 2011), we avoided using sham
185 stimulation.

186 Soleus H-reflex, lower (AH) and upper (ADM) limb MEPs and F-wave were recorded
187 before tsDCS (baseline), immediately after tsDCS offset (T0), and at 30 min after tsDCS
188 offset (T30); all tests were performed in the same order as we have listed them above. For all
189 the electrophysiological recordings we chose the left side to avoid any possible modification
190 on motor response on the right side due to the current flowing through the reference tsDCS
191 electrode possibly acting on peripheral nerves. During each tDCS session, subjects were
192 interviewed to assess the general tolerability of the procedure, and were asked to report any
193 adverse effect, particularly itching, tingling, burning, and pain sensations.

194

195 *Data analysis*

196 We analyzed statistical significant changes for the following variables: MEP threshold
197 (percentage of the maximum stimulator output), MEP area (mVms), MEP latency (ms); H-
198 reflex threshold, H-reflex latency (ms), H-reflex amplitude (μ V), and Hmax/CMAPMax
199 amplitude ratio; F-wave mean latency (ms), minimal latency (ms), amplitude (μ V) and
200 temporal dispersion (ms). All the neurophysiological measures were considered as
201 independent variables and were analyzed separately. Each variable is expressed throughout
202 the text as a percentage of baseline values (= 100%), after anodal and cathodal tsDCS, at T0
203 and T30.

204 First, to verify the absence of biases due to intra-subject changes across tsDCS
205 sessions, a t-test was run to compare baseline data for anodal and cathodal tsDCS. Values of
206 $p < 0.05$ were considered to indicate statistical significance.

207 Then, tsDCS-induced changes in each variable were tested with a two-way repeated
208 measure analysis of variance (ANOVA) (STATISTICA 5.5, StatSoft Inc.) with main factors
209 “stimulation”, two levels (anodal and cathodal), and “time”, three levels (B, T0 and T30).
210 Bonferroni corrected t-tests were used for *post hoc* comparison ($p < 0.025$). Values in the text
211 and figures are expressed as mean \pm SEM.

212

213 **Results**

214 All participants tolerated the procedure well and none of them reported adverse effects.
215 Participants occasionally referred a slight tingling or itching sensation below the stimulating
216 electrodes (not distinguishable between the two polarities) that disappeared within few
217 seconds or after wetting the electrode sponges.

218

219 *tsDCS effects in the lower limb muscles*

220 No differences were found between anodal and cathodal tsDCS in baseline values for
221 any of the measured variables in the lower-limb muscles. Neither anodal nor cathodal tsDCS
222 induced changes in H-reflex and F-wave variables (Table 1).

223 Though MEP latency showed an un-specific effect of time (two-way ANOVA, factor
224 “time”: $p = 0.009$) (baseline latency values were in general shorter than latency values at T30
225 (*post-hoc* analysis: baseline vs T30 $103.53 \pm 0.95\%$, $p = 0.008$), MEP latency was not affected
226 by tsDCS (two-way ANOVA, factor “stimulation”: $p = 0.83$), nor by the interaction between
227 tsDCS and time (two-way ANOVA, interaction “stimulation x time”: $p = 0.85$).

228 RMT was affected by tsDCS (two-way ANOVA, factor “stimulation”: $p = 0.012$;
229 factor “time” $p = 0.11$; interaction “stimulation x time”, $p = 0.011$). More specifically, after
230 anodal tsDCS (Figure 1A) RMT increased (*post hoc* analysis anodal tsDCS: T0_A vs. B_A:
231 $107.33 \pm 3.3\%$, $p = 0.006$; T30_A vs. B_A: $108.37 \pm 3.2\%$, $p = 0.002$), whereas after cathodal

232 tsDCS (Figure 1B and Figure 2A) it remained unchanged (*post hoc* analysis, cathodal tsDCS:
233 T0_C vs. B_C: $96.83 \pm 2.3\%$, $p = 0.15$; T30_C vs. B_C: $100.60 \pm 2.1\%$, $p = 0.8$).

234 MEP area was also modulated after tsDCS (two-way ANOVA, factor “stimulation”: p
235 = 0.96; factor “time” $p=0.78$ interaction “stimulation x time”, $p=0.008$) (Figure 2B): whereas
236 after anodal tsDCS MEP area failed to change (*post hoc* analysis anodal tsDCS: T0_A vs. B_A:
237 $66.09 \pm 9.1\%$, $p = 0.24$; T30_A vs. B_A: $58.12 \pm 10.48\%$, $p = 0.13$), after cathodal tsDCS MEP
238 area increased (*post hoc* analysis cathodal tsDCS: T0_C vs. B_C: $139.71 \pm 12.9\%$, $p = 0.018$;
239 T30_C vs. B_C: $132.74 \pm 22.0\%$, $p = 0.02$).

240 In conclusion, anodal tsDCS increases RMT whereas cathodal tsDCS increases the
241 MEP area in lower limb muscles.

242

243 *tsDCS effects in the upper limb muscles*

244 No differences were found between anodal and cathodal tsDCS in baseline values for
245 any of the measured variables in the upper-limb muscles. Neither anodal nor cathodal tsDCS
246 induced changes in F-wave variables (Table 1).

247 The two-way ANOVA disclosed no anodal or cathodal tsDCS-induced effect on MEP
248 variables (Figure 1C and 1D, Figure 2C and 2D). RMT did not change over time and across
249 sections (Figure 2C: two-way ANOVA, factor “stimulation”: $p = 0.54$; factor “time” $p=0.14$;
250 interaction “stimulation x time”, $p=0.48$), nor it did MEP area (Figure 2D: two-way ANOVA,
251 factor “stimulation”: $p = 0.58$; factor “time” $p=0.63$; interaction “stimulation x time”, $p=0.23$).
252 MEP latency was not affected by stimulation (two-way ANOVA, factor “stimulation”: $p =$
253 0.26 ; interaction “stimulation x time”, $p=0.29$) but showed the unspecific time-related
254 increase at T30 compared to baseline (two-way ANOVA, “time” $p=0.013$; *post-hoc* analysis:
255 baseline vs T30 $103.53 \pm 0.95\%$, $p=0.011$).

256

257

258

259 **Discussion**

260 Whereas tsDCS leaves H-reflex, F-wave and upper-limb RMT and MEPs size
261 unchanged, it modulates the excitability in corticospinal projections to lower limb muscles for
262 at least 30' after stimulation offset, inducing polarity-dependent excitability changes: anodal
263 tsDCS significantly increases RMT, whereas cathodal stimulation increases MEP area.

264 The absence of F-wave changes in our experiments therefore argues against the
265 occurrence of changes in postsynaptic motorneuronal excitability after tsDCS. The absence of
266 H/M ratio changes in this and a previous study from our group (Cogiamanian et al. 2011)
267 agrees with previous observations (Lamy et al. 2012; Winkler et al. 2010).

268 The tsDCS-induced corticospinal excitability changes at lower limb level are in line
269 with our previous observation that tsDCS modulates conduction along human spinal
270 ascending pathways (Cogiamanian et al., 2008; Truini et al., 2011) and are consistent with the
271 effects of anodal spinal DC on motor potentials elicited by cortical stimulation in the mouse
272 *triceps surae* (Ahmed, 2011).

273 How tsDCS influences the corticospinal system remains hypothetical. First, because
274 tDCS influences neurotransmitters in the brain (Rango et al. 2008), tsDCS could do the same
275 in the human spinal cord, ultimately modulating the corticospinal output as in animals
276 (Ahmed and Wieraszko 2012). As recently shown in animals (Ahmed, 2013), cathodal tsDCS
277 can amplify segmental responses to supraspinal drive by increasing glutamate release at the
278 spinal level, although mice typically lack the monosynaptic corticomotoneuronal synapse of
279 higher primates. Another possibility is that tsDCS could influence neural activity in ascending
280 spinal pathways, ultimately modulating the excitability in their cortical targets including the
281 motor areas, as changes in RMT suggest. Possible support for a cortical mechanism comes

282 from a report that invasive spinal stimulation seems to modulate intracortical facilitation
283 (Schlaier et al. 2007). Thus, our data expand, rather than be simply contradictory, previous
284 knowledge on putative tsDCS targets, including supra-spinal, and possibly polarity-specific,
285 effects of spinal current polarization; this possibility also agree with recent evidence in rats
286 that non-invasive spinal stimulation modulates the activity of gracile nucleus and primary
287 somatosensory cortex (Aguilar et al. 2011).

288 Finally, tsDCS could influence the conductive properties of the corticospinal tract, for
289 example by decreasing/increasing the number of axons conducting an action potential. For
290 instance, anodal tsDCS can induce a hyperpolarizing conduction block thus blocking action
291 potentials along the pyramidal tract (Bhadra and Kilgore 2004). However, given that we used
292 low current intensities, anodal block could not be the sole explanation for the effects of anodal
293 tsDCS; moreover, it's known that, also in routinary electrodiagnostic testings, geometry and
294 tissue distribution of electrical fields are additional critical parameters for inducing a
295 hyperpolarizing block (Dreyer et al. 1993; Kirshblum et al. 1998).

296 Although the mechanisms underlying the tsDCS-induced changes in the corticospinal
297 system remain speculative, our finding that anodal tsDCS seems mainly to affect the RMT
298 whereas cathodal tsDCS predominantly influences MEP area is intriguing. A possible
299 explanation is that the mechanisms underlying cathodal and anodal tsDCS differ and could
300 have a different putative circuit(s)-pathway(s)-neurotransmitters, i.e. anodal current might
301 preferentially act on one target system, whereas cathodal current acts on another. This
302 possibility agrees with evidence that anodal and cathodal brain tDCS act through different
303 brain neurotransmitters: for instance, whereas anodal tDCS reduces gamma aminobutyric acid
304 (GABA), cathodal tDCS decreases glutamate (Stagg et al. 2009). Increase in MEP area
305 following cathodal tsDCS is in line with previous reports (Aguilar et al. 2011; Ahmed 2013;
306 2011; Alanis 1953; Eccles et al. 1962) and agree with data in animals showing an increased

307 recruitment of larger motor units following cathodal polarization (Ahmed and Wieraszko
308 2012); as suggested in a recent work by our group, cathodal, but not anodal, stimulation could
309 have a trans-synaptic effect mediated by spinal interneurons likely involving Renshaw cells
310 network (Bocci et al. 2014).

311 Among methodological issues related to our study the first is that to avoid subjecting
312 participants to another experimental session, we decided not to test them under sham
313 conditions. Sham testing was also unnecessary given that even though changes induced by
314 cathodal tsDCS and anodal tsDCS go in opposite directions, subjects cannot distinguish
315 between the two polarities. We therefore considered each polarity as the best possible control
316 for the other as previously reported (Cogiamanian et al. 2008; Lamy and Boakye 2013a;
317 Truini et al. 2011). Besides, as recently showed by Kessler and colleagues (Kessler et al.
318 2012), sham stimulation may be an inappropriate control condition for some studies, because
319 sensory side effects seem to be more frequent and severe in active than in sham tDCS.

320 A second methodological issue is about possible pitfalls concerning the use of H-
321 reflex and F-wave to assess spinal motorneuron excitability. The absence of H-reflex changes
322 suggests no modification to small motorneurons and the lack of F-wave effects rules out
323 changes in large motorneurons (McNeil et al. 2013). However, the classical view that H-
324 reflex and F-wave represent separate and complementary events at spinal level, i.e.
325 presynaptic inhibition versus changes in intrinsic motorneuron excitability (Fisher 1992; Leis
326 et al. 1995), is questionable. In fact, while H-reflex could be also affected by post-activation
327 depression and changes in axonal excitability itself (McNeil et al. 2013; Pierrot-Deseilligny et
328 al. 1981), many reports have suggested that F-waves offers only a flawed measure of
329 motorneuron excitability (Hultborn and Nielsen 1995).

330 A further important point is to compare our data with those by Lim and Shim (Lim and
331 Shin, 2011) who found that cervical spinal DC stimulation influences upper-limb MEPs. But

332 they did not report polarity-specific effects. Their results cannot be compared with ours
333 because they studied a different anatomical region, with a different recording montage and
334 stimulation intensity, and used smaller electrodes. Finally, there are known features in how
335 corticospinal excitability differs between the upper and lower body, which may account, at
336 least in part, for differences between our results and data obtained by Lim and Shin.
337 Particularly, monosynaptic corticomotorneuronal projections, with fast conducting motor
338 units, are more prominent for hand than for proximal arm or lower limb muscles (Brouwer
339 and Ashby 1992; Dalpozzo et al. 2002; Palmer and Ashby 1992); in this view, lack of changes
340 in MEPs amplitude following anodal tsDCS may be caused by the greater desynchronization
341 of corticomotorneuronal input compared with upper limb muscles, related to the involvement
342 of fibers with different sizes or activation of polysynaptic descending pathways.

343 Overall, our data support the conclusion that tsDCS induces changes in corticospinal
344 tract excitability. The first clinical observations with tsDCS are encouraging: Hubli (Hubli et
345 al., 2013) found that anodal tsDCS can improve gait in patients with spinal cord injury.
346 Together with a previous report describing how tsDCS influences ascending spinal pathways
347 (Aguilar et al., 2011), the present experiments suggest that the spinal cord could act as a
348 “highway” for conveying tsDCS-induced changes to the brain thereby inducing
349 suprasegmental effects in the brain and brainstem. Because tsDCS is simple, safe and non-
350 invasive, our observation opens the way to new approaches using this technique in widely
351 ranging neurological conditions characterized by corticospinal and spinal cord dysfunction
352 and possibly even in brain disorders.

353

354

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360 of interest.

361

362 **Figure Legends**

363 *Figure 1. A, B* - Lower-limb motor evoked potentials (MEPs) before (baseline, B),
 364 immediately after (T0) and at 30 min (T30) after anodal (A) or cathodal (B) tsDCS in a
 365 representative subject. Horizontal calibration 10 ms; vertical calibration 1 mV; each trace is
 366 the superimposition of five sweeps. The table on the right side reports the average values of
 367 RMT, MEP area, and MEP latency for the represented subject. Note that whereas anodal
 368 tsDCS decreased the MEP area at T0 and T30, cathodal tsDCS increased the MEP area.
 369 Vertical arrows represent the stimuli. *C, D* - Upper-limb motor evoked potentials (MEPs)
 370 before (baseline, B), immediately after (T0) and at 30 min (T30) after anodal (C) or cathodal
 371 (D) tsDCS in a representative subject. Horizontal calibration 10 ms; vertical calibration 0.5
 372 mV; each trace is the superimposition of five sweeps. The table on the right side reports the
 373 average values of RMT, MEP area, and MEP latency for the represented subject. Note that
 374 neither anodal nor cathodal tsDCS affected MEPs. Vertical arrows represent the stimuli.

375

376 *Figure 2. A, B* - Effects of transcutaneous spinal direct current stimulation (tsDCS) on resting
 377 motor threshold (A, RMT) and motor evoked potentials (B, MEP) area when responses were
 378 recorded from abductor hallucis muscle (AH; data are expressed as % of baseline). Group
 379 data are presented as mean \pm SEM changes induced by anodal (gray) or cathodal (dark gray)
 380 tsDCS immediately after current offset (T0) and 30 minutes later (T30; error lines are
 381 standard error of the mean, SEM). Note that anodal and cathodal tsDCS induced significantly
 382 different, opposite changes in RMT and MEP area. *C, D* - Effects induced by tsDCS on RMT
 383 (C) and MEP size (D) when responses were recorded from abductor digiti minimi (ADM)
 384 muscles. Group data are presented as mean \pm SEM changes induced by anodal (gray) or
 385 cathodal (dark gray) tsDCS immediately after current offset (T0) and 30 minutes later (T30;

386 error lines are standard error of the mean, SEM). Neither anodal nor cathodal tsDCS
387 significantly changed RMT or MEP area. ** = $p < 0.05$.

388

389 *Table 1* -Changes in F-wave and H-reflex variables over time (B, T0, T30), in both
390 experimental conditions (anodal and cathodal stimulation). We found no significant changes
391 in F-wave and H-reflex variables across the different time points. p values refer to two-way
392 repeated measures ANOVA with “stimulation” and “time” as factor (interaction effects). Data
393 are shown as mean values \pm SEM. Minimal, mean latencies and temporal dispersion are
394 expressed in ms, H-reflex area as mVms.

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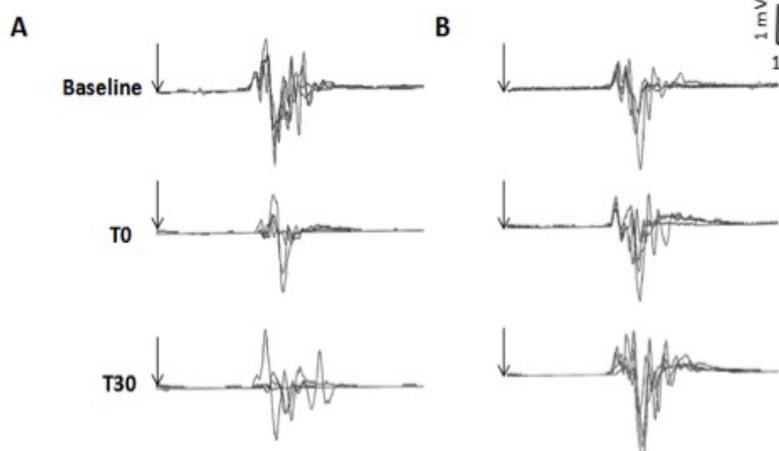
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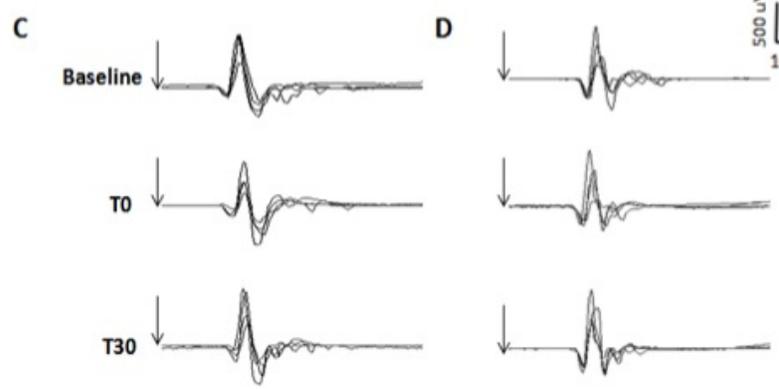
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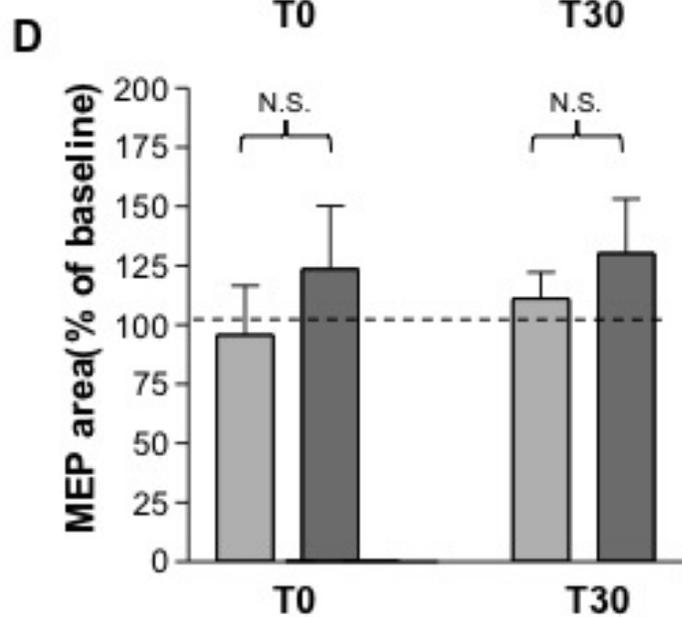
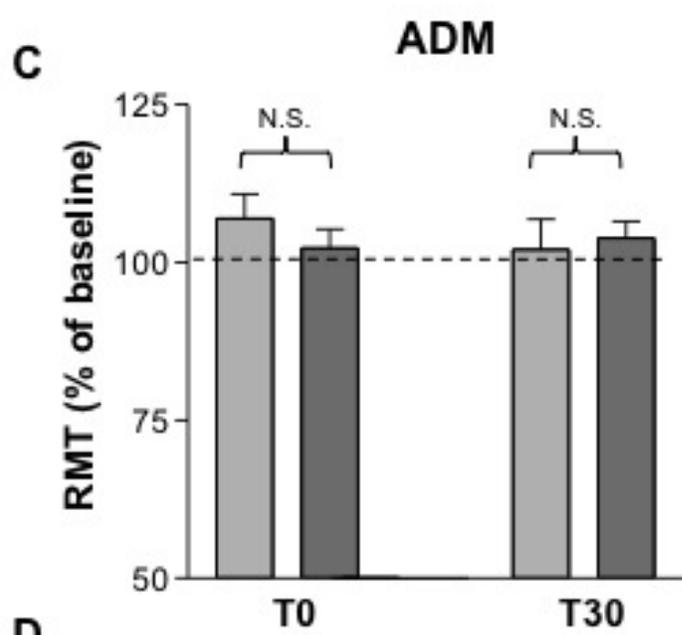
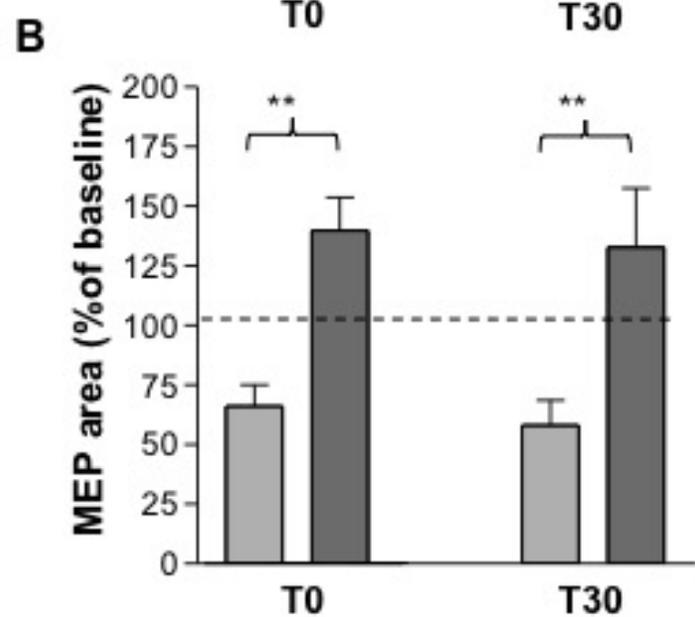
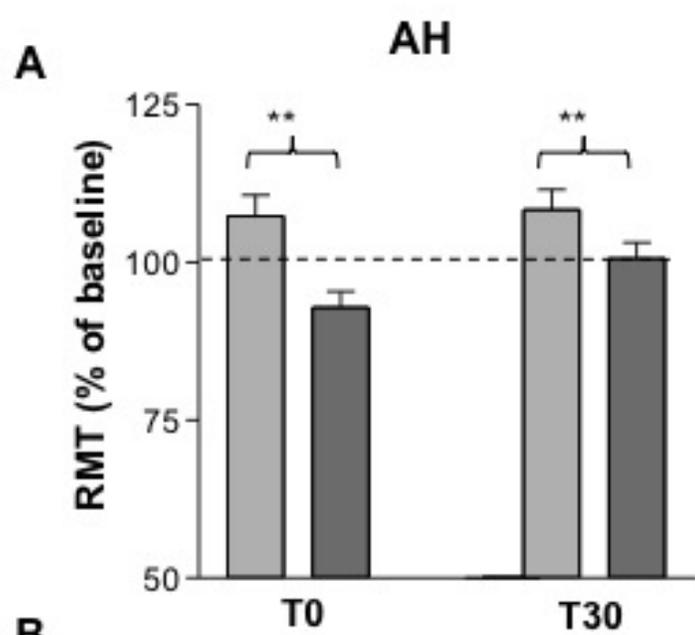
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| LOWER LIMB | RMT (%) | | MEP area (mV) | | MEP Latency (ms) | |
|------------|--------------|---------------|---------------|---------------|------------------|---------------|
| | Anodal tsDCS | Catodal tsDCS | Anodal tsDCS | Catodal tsDCS | Anodal tsDCS | Catodal tsDCS |
| Baseline | 60 | 55 | 3.11 | 2.09 | 33.68 | 34.85 |
| T0 | 75 | 60 | 1.42 | 2.95 | 38 | 37.8 |
| T30 | 75 | 60 | 1.63 | 3.75 | 36.93 | 36.9 |



| UPPER LIMB | RMT (%) | | MEP area (mV) | | MEP Latency (ms) | |
|------------|--------------|---------------|---------------|---------------|------------------|---------------|
| | Anodal tsDCS | Catodal tsDCS | Anodal tsDCS | Catodal tsDCS | Anodal tsDCS | Catodal tsDCS |
| Baseline | 55 | 50 | 1.15 | 1.08 | 18.8 | 20.6 |
| T0 | 55 | 50 | 1.23 | 1.28 | 20.5 | 19.6 |
| T30 | 55 | 50 | 1.2 | 1.36 | 19.3 | 20.5 |



■ Anodal
■ Cathodal

| | | F-wave | | | | | H-reflex | | | | |
|----------------------------------|------------|-----------------------------|--------------------------|------------------------|-----------------------------|--------------------------|------------------------|-----------------------|---------------------|--------------------|------------------|
| | | AH | | | ADM | | Soleus | | | | |
| | | <i>Minimal Latency (ms)</i> | <i>Mean Latency (ms)</i> | <i>Dispersion (ms)</i> | <i>Minimal Latency (ms)</i> | <i>Mean Latency (ms)</i> | <i>Dispersion (ms)</i> | <i>Threshold (ms)</i> | <i>Latency (ms)</i> | <i>Area (mVms)</i> | <i>Ratio H/M</i> |
| Anodal (mean ± SEM) | <i>B</i> | 46.4 ± 1.3 | 48.2 ± 1.5 | 4.3 ± 0.6 | 25.8 ± 0.6 | 27.9 ± 0.8 | 2.9 ± 0.5 | 14.7 ± 3.5 | 28.9 ± 1.0 | 7.4 ± 1.0 | 0.4 ± 0.1 |
| | <i>T0</i> | 47.0 ± 1.5 | 49.3 ± 1.4 | 4.3 ± 0.5 | 26.2 ± 1.0 | 27.8 ± 0.8 | 2.8 ± 0.7 | 13.9 ± 2.8 | 28.3 ± 1.3 | 7.1 ± 0.6 | 0.4 ± 0.05 |
| | <i>T30</i> | 47.2 ± 1.4 | 50.5 ± 2.5 | 4.4 ± 0.7 | 26.3 ± 0.9 | 28.3 ± 1.9 | 3.0 ± 0.4 | 13.9 ± 1.8 | 29.3 ± 1.4 | 7.7 ± 0.9 | 0.4 ± 0.13 |
| Cathodal (mean ± SEM) | <i>B</i> | 46.3 ± 1.2 | 48.6 ± 1.2 | 4.4 ± 0.5 | 25.3 ± 1.2 | 27.8 ± 1.5 | 3.0 ± 0.7 | 14.1 ± 3.0 | 28.0 ± 1.2 | 7.7 ± 0.6 | 0.4 ± 0.04 |
| | <i>T0</i> | 46.3 ± 1.4 | 48.8 ± 1.7 | 4.7 ± 0.3 | 25.5 ± 1.5 | 28.0 ± 1.0 | 3.1 ± 0.4 | 14.1 ± 2.5 | 27.8 ± 1.3 | 7.6 ± 1.1 | 0.4 ± 0.1 |
| | <i>T30</i> | 47.2 ± 1.6 | 49.1 ± 1.5 | 4.8 ± 0.7 | 26.0 ± 1.1 | 28.3 ± 1.0 | 2.8 ± 0.6 | 13.8 ± 2.7 | 28.9 ± 0.8 | 7.9 ± 1.4 | 0.3 ± 0.3 |
| p-value | | <i>0.6</i> | <i>0.2</i> | <i>0.8</i> | <i>0.3</i> | <i>0.9</i> | <i>0.3</i> | <i>0.8</i> | <i>0.7</i> | <i>0.8</i> | <i>0.3</i> |